IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :
Kristian BERG et al. :
Serial No. 09/524,454 : Examiner: G.R. Ewoldt
Filed: March 10, 2000 : Group Art Unit: 1644
For: METHOD OF EXPRESSING :
ANTIGENS ON THE SURFACE OF :
ANTIGEN PRESENTING CELLS :
BY PHOTOCHEMICAL :
INTERNALIZATION :

DECLARATION UNDER RULE 132

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

I, Pål Kristian Selbo, a Norwegian citizen of Fredrikke Qvamsgate 9, 0172 Oslo, Norway,

declare as follows:

1. I am a postdoctoral fellow working in the laboratory of the inventor Kristian Berg. Under the instruction of Kristian Berg I conducted the experiment which now forms Example 3 of the present application.

2. Relevant copies of pages from my laboratory notebook which documents the results of this experiment are attached as Exhibit 1. In the table on the first page, the first column shows the times of illumination. The results marked "1" are denoted "Total" and correspond to intact cells. The results marked "2" are not further labelled, but correspond to the cell culture after electropermeabilisation but before fractionation into cytosol and cell corpses. The results marked "3" are denoted "Cytosol" and correspond to extracted cytosol. The results marked "4" are denoted "Pellet" and correspond to cytosol-free cell corpses separated from the cytosol by electropermeabilisation and density centrifugation. The levels of horseradish peroxidase (HRP)

activity in these fractions are reflected by the OD_{450} measurements shown in the second column. These were standardized relative to the "Total" value at zero time (these standardized values are shown in the right hand side of the right column).

3. These results are shown graphically on the next page of the laboratory notebook for the total, cytosol and pellet fractions (Exhibit 1). The total fraction which is shown with filled circles shows full HRP activity at time zero which drops off as the time of irradiation increases. The cytosol fraction shown by filled squares shows that activity increases with illumination time as the endocytosed HRP is released into the cytosol. The pellet fraction or cell corpses are denoted by filled triangles and show that HRP activity reduces in this fraction as a function of illumination time as the HRP is transferred to the cytosol.

4. Figure 4 presented in the current application corresponds to the figure shown in the Exhibit. The legend however erroneously transposes the cytosol and intact cell fractions. This is clearly in conflict with the original which is correctly shown in the laboratory notebook and shows that the mix-up occurred at a later stage.

5. It is evident from the text that an error has occurred since Example 3 refers to the release of a large fraction of endocytosed HRP into the cytosol by photochemical internalisation which must correspond to the line of the graph which reaches >60% HRP activity relative to the intact cells after light exposure, ie. the line currently labelled with hollow circles which should therefore be denoted in the legend as the cytosol fraction. Since the cytosol and cytosol-free cell corpses are together components of the intact cells, it can only follow that the filled circles must correspond to the intact cells and the filled triangles of Figure 4 must correspond to the cytosol-free cell corpses.

6. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made

with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Codes, and that such wilful false statements may jeopardize the validity of the application and any patent issuing thereon.

Pål Kristian Selbo ..... 6/3-03 .....

Pål Kristian Selbo Date

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EXHIBIT 1

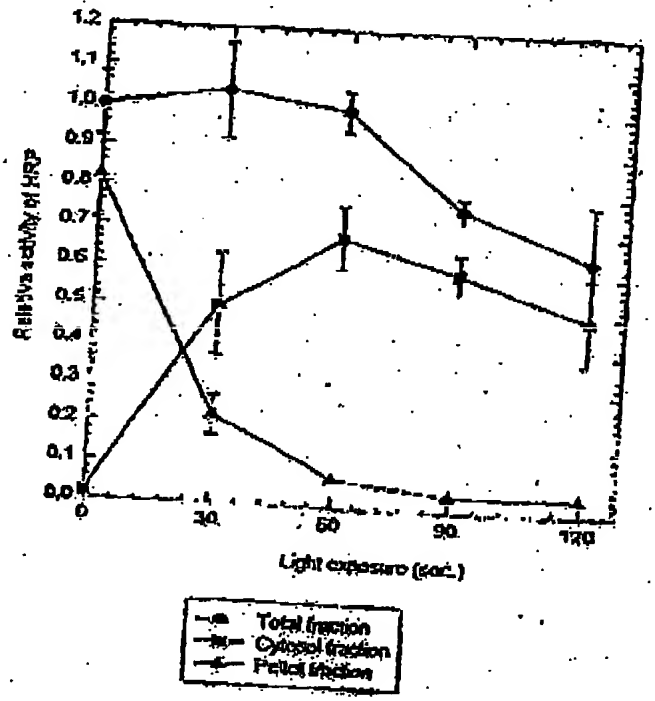
HRP-aktivitet i 3025-celler / i ulike cellefraksjoner etter PDT-beh. og elektropermeabilisering.

Std. prosedyre ble fulgt. (se s. 378). Denne gangen ble det gitt lysdose fra 0-120 sek (120 sek ≈ D_{10})

FORSØK: PDT/HRP / elektroperm.

	PRØVE		OD 450	% akt.	rel.
Total	(1)	0	0,45	0,06	1
		30	0,47	[illegible]	1,04
		60	0,45	[illegible]	1
		90	0,34	0,63	0,76
		120	0,29	[illegible]	0,64
	(2)	0	0,58		
		30	0,55		
		60	0,49		
		90	0,41		
		120	0,37		
Cytosol	(3)	0	0,01	[illegible]	0,02
		30	0,23		0,51
		60	0,31		0,68
		90	0,32	0,66	0,68
		120	0,22		0,45
Lekk.	(4)	0	0,38		0,82
		30	0,10		0,13
		60	0,03		0,07
		90	0,02		0,04
		120	0,02		0,04

Relative activity of HRP in different cell fractions after PDT treatment followed by electropermeabilisation of NHIK 3025 cells. 18 hrs. coincubation of 1,0 mg/ml HRP and 3,2 µg/ml $TPPS_{2a}$. 12/12-96.



PCI on HEP 1.